Pre-mRNA processing: **Insights from nonsense**

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In eukaryotic cells, translation is thought to be confined to cytoplasm, but two recent studies have challenged this notion, one showing that an mRNA's open reading frame influences nuclear events as early as release from the site of transcription, and the other by providing evidence for protein synthesis within the nucleus.

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Nonsense mutations are nucleotide substitutions that change a coding triplet into a premature termination codon, resulting in premature translation termination. Because of their properties, nonsense mutations played a key role in the early days of molecular biology; for example, they were essential for deciphering the genetic code. Now, nonsense mutations are once again becoming a hot topic of research in the study of gene expression. The reason for this interest is that the study of this class of mutations, together with other recent observations, is revealing unexpected links between translation and mRNA metabolism [1,2].

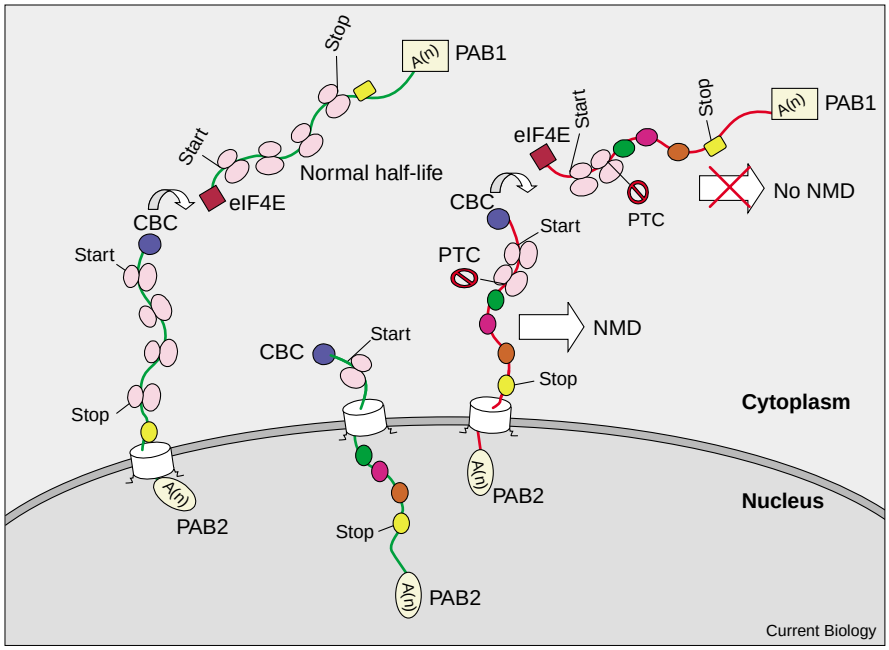
The presence of a premature termination codon can lead to a dramatic reduction in mRNA levels. It is generally accepted that this phenomenon constitutes an evolutionarily conserved 'quality control' or 'mRNA surveillance' mechanism, which protects cells from the potentially deleterious effects of truncated proteins. This mechanism is referred to as nonsense mediated mRNA decay (NMD) [2–6]. The recognition of premature termination codons has been assumed to occur during cytoplasmic translation, and premature translation termination is thought to activate a specific protein complex — the surveillance complex — which triggers accelerated decay of the aberrant mRNA [7].

Contrary to the expectation that recognition of premature termination codons should occur exclusively during cytoplasmic translation, many studies in mammalian cells showed that NMD actually occurs while the mRNA is still associated with the nucleus (reviewed in [3]). A common explanation for these observations is that recognition of premature termination codons occurs preferentially during the first round of translation, before the mRNA has completely exited the nuclear pore. For most mammalian genes, after the mRNA is completely released from the nucleus it becomes refractory to NMD [3]. In agreement with this model (Figure 1), it was recently reported that translation can occur while the mRNA is bound by the nuclear cap-binding complex (CBC), which recognises the mRNA's characteristic 5′ 'cap' structure. Furthermore, mRNAs containing premature termination codons are preferentially associated with the CBC and not with the translation initiation factor eIF4E, a cytoplasmic cap-binding protein [8]. Both CBC and eIF4E can bind the translation initiation factor eIF4G, which plays an essential role in translation initiation by recruiting the small ribosomal subunit [9,10].

If 'pioneer' rounds of translation occur on mRNAs as they emerge from the nucleus, it is possible that aspects of nuclear NMD are merely a consequence of recognition of premature termination codons at the cytoplasmic side of the nuclear envelope [8,11]. Not all the data from mammalian systems are so easily explained, however. For example, several reports show that a premature termination codon can directly affect precursor RNA (pre-mRNA) splicing [12–15], or both pre-mRNA 3′ end processing and splicing [16]. The observation that a premature termination codon can affect co-transcriptional events suggests the possibility that open readings frames (ORFs) are recognised inside the nucleus. There are alternative interpretations, however, and the existence of nuclear ORF recognition is still a very debatable issue [11].

A recent paper has made a significant contribution to this debate. Muhlemann *et al.* [1] report convincing evidence that the destruction of an ORF can influence events at or near the site of transcription. They studied, in mammalian cells, transcipts of immunoglobulin μ and T cell receptor (TCR) β genes either containing (PTC^+) or lacking (PTC^-) a premature termination codon. Essentially all PTC^+ mutants in this system lead to a drastic reduction in the total cellular level of the mRNA. The authors used fluorescent *in situ* hybridization (FISH) to localize transcripts: this revealed a bright nuclear spot corresponding to the gene locus, presumably because the local concentration of a transcript is highest at its site of transcription. Unexpectedly, cells carrying PTC^+ alleles exhibit spots that are larger and brighter than those of PTC^- alleles. As similar results were obtained using an intronic probe, Muhlemann *et al.* [1] conclude that the increased fluorescence represents unspliced transcripts accumulating at or near the site of transcription. Consistent with this interpretation, RNA analysis reveals that unspliced RNA levels are increased about five-fold in the presence of a premature termination codon, despite a reduction in mRNA levels.

Figure 1



A cartoon of the pioneer translation model. Normal mRNAs (PTC$^-$), on the left, are represented as green lines, and PTC$^+$ mRNAs on the right are represented by red lines. mRNA molecules exit the nucleus as an mRNP decorated with a number of factors (represented by different colour ovals), including hnRNPs proteins, post-splicing factors and mRNA export factors [26]. All mRNAs are also likely associated with nuclear cap binding complex (CBC, blue circle) and poly(A) binding protein 2 (PAB2). As the 5′ end of the mRNP emerges from the nuclear pore (represented by a tunnel) it associates with ribosomes (pink) and translation is initiated. During translation, some proteins (ovals) are stripped off the mRNP, while others are recruited. For example, CBC and PAB2 are exchanged for eIF4E (red square) and PAB1 (light yellow). In the presence of a premature termination codon, the ribosome is unable to displace factors (ovals) which cause NMD while the mRNA is still nuclear-associated [26]. NMD susceptibility lasts while the CBC remains associated with the mRNA; after CBC has exchanged with eIF4E, the mRNA becomes resistant to NMD [8].

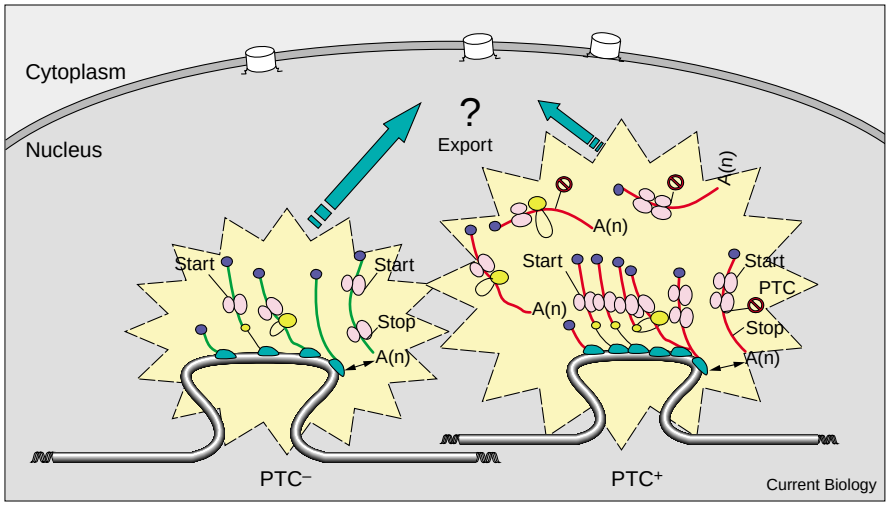
As it is established that efficient pre-mRNA splicing is affected by flanking exonic sequences, Muhlemann *et al.* [1] used combinations of mutations to test whether the nucleotide changes *per se*, or disruption of the open reading frame, affected splicing. They found that a missense mutation cannot substitute for a premature termination codon. Further, an out-of-frame 10 nucleotide insertion that generates a premature termination codon resulted in both NMD and an increase in pre-mRNA levels, whereas an in-frame 9 nucleotide insertion did not. Combining the 10 nucleotide insertion with a downstream 1 nucleotide deletion that reestablishes the ORF completely reverted both mRNA and pre-mRNA levels to those of wild type. These observations imply that, at least in the case of TCR β transcripts, nonsense mutations cause an increase pre-mRNA levels as a consequence of altering the open reading frame and not from disrupting splicing regulatory elements.

The simplest interpretation for the results of Muhlemann *et al.* [1], as well as others reported previously [12–16], is that functional ribosomes are present in the nucleus. While it has been assumed that translation-competent ribosomes are absent from the nucleus, ribosomal subunits are synthesized and assembled in the nucleolus, and the nucleus probably abounds with charged tRNAs and translation factors [17–19]. Furthermore, translation on CBC-bound transcripts might indicate that nuclear mRNA is translation-competent.

In agreement with this simple interpretation is a recent study by Ibora *et al.* [2], showing that translation can occur in a number of intranuclear foci in mammalian cells. The authors incubated permeabilized cells or isolated nuclei with labelled aminoacylated tRNAs. By indirect immunofluorescence they detect amino acid incorporation at discrete sites within the nucleus. Furthermore, electron microscopy indicated that these translation sites localise close to sites of active transcription. This study, again with the persuasion of microscopy, appears to validate a number of papers from the 1960s and 1970s reporting the isolation of nuclear polysomes (reviewed in [20–22]). Evidence for nuclear polysomes was also found in a recent study on the slime mold *Dictyostelium discoideum* [23].

Taken together, the data of Muhlemann *et al.* [1] and Ibora *et al.* [2] provide strong evidence that open reading frames are recognised in discrete locations — spots — within the nucleus. The exact nature of these spots is still a mystery. For example, they may correspond to single transcription units, or to ‘factories’ where several genes are transcribed and processed together [24]. Recent work on budding yeast indicates that polyadenylated transcripts may also transiently accumulate near sites of transcription [25]. Within these spots, ribosomes could be translating mature mRNA, released pre-mRNA or nascent transcripts (Figure 2). In the presence of a premature termination codon, this transient accumulation of RNA would be increased, causing the larger spots seen by RNA FISH.

Figure 2



A cartoon of the region near the transcription site – an example of the 'spots' referred to in the text. The drawing on the left is a wild-type (PTC$^-$) locus, and a PTC$^+$ locus is on the right. In both cases, as the 5′ end of the nascent transcript emerges from the transcription machinery (green) it associates with CBC and other factors (see Figure 1), including splicing commitment factors (small yellow circle) and spliceosomes (big circles). In the case of wild-type genes most of the RNA in the spot is nascent, as splicing occurs co-transcriptionally and the transcript rapidly leaves the spot after cleavage and polyadenylation. The data discussed in the text suggest that ribosomes (pink) are also recruited to the nascent mRNP and to transcripts still associated with the spot. In the case of PTC$^+$ genes, recognition of a premature termination codon delays the release on the mRNA from the template and/or from the spot. This delay causes an increase in nascent transcript and leads to the retention of both mRNA and pre-mRNA in the spot. When the transcript leaves the spot it is committed to nuclear export. Whether it remains associated with ribosomes as it is released from the spot is not known.

The increase in pre-mRNA levels caused by a premature termination codon suggests that translation can occur on pre-mRNA, but the puzzling observation is that premature termination codons can also lead to retention of upstream introns. To explain this, Muhlemann *et al.* [1] suggest that the ribosome scanning and the recognition of premature termination codons occurs on spliced transcripts which are still associated with the site of transcription, and that this results in inhibition of splicing of nearby pre-mRNA.

The distinction between ribosomes scanning spliced transcripts or pre-mRNA molecules is an important one. If the latter were correct, the prediction would be that there must be a direct link between ribosome scanning of the ORF, intron recognition and splicing. This distinction is also important for understanding whether nuclear NMD is simply a consequence of inhibiting pre-mRNA processing, or whether NMD and pre-mRNA effects are two distinct consequences of nonsense mutations.

In conclusion, the developments reviewed above provide strong evidence that ribosomes can recognize the ORFs in nuclear RNAs. More experiments, and possibly new experimental approaches, will be required to understand the link between nuclear translation and pre-mRNA processing. It will also be important to determine whether nuclear scanning is a general feature of gene expression or a curious feature of a few 'odd' genes.

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